II. **REMARKS**

Claims 1, 3-15, and 17-52 are pending. Claims 11, 12, 15, 17, 21-22, and 26-52 are withdrawn. Claims 1, 3, 14, and 19 are amended and claims 2, 16, and 53 are canceled. The amendments are supported by the originally filed specification and claims. In particular, the amendments to claims 3, 14 and 19 are supported, for example, by originally filed claims 3, 14, 16, and 19. Meanwhile, the amendment to claim 1 is supported, for example, by originally filed claim 2 and Example 10 in the specification. No new matter is added.

Claims 14 and 19 are objected for containing non-elected subject matter. Applicants respectfully submit that these objections are overcome by the above amendments to claims 14 and 19. Accordingly, Applicants respectfully request reconsideration and withdrawal of the objections to claims 14 and 19.

Claim 3 is rejected under 35 U.S.C. § 112, first paragraph for asserted indefiniteness. This rejection is traversed.

Present claim 3, which was amended in order to expedite prosecution, discloses "a carrier protein having: (i) a stable polyamine acid extension and (ii) intact biological functions of a corresponding carrier protein." As such, present claim 3 clearly indicates that the modified carrier protein of the presently claimed invention should retain the biological function of the native unmodified protein. Further, Applicants note that the specification discloses the following:

> "Carrier protein" in the present invention means a protein, which can be stably extended by a polyamino acid sequence or a peptide. The nucleotide sequence encoding the functionally intact carrier protein is fused in-frame with a nucleotide cassette comprising one

> or more selected codons encoding amino acid residues. The inserted codons encoding the desired amino acid residues may not disturb the normal biological functions of the carrier protein as compared with the corresponding native unmodified protein.

(Specification, page 13, lines 18-23) (emphasis added).

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claim under 35 U.S.C. § 112, first paragraph for asserted indefiniteness.

Claims 1, 3, 6-10, 18, and 23-25 are rejected under 35 U.S.C. 102(b) as being anticipated by Hoffman (U.S. Patent No. 5,003,045). This rejection is traversed.

Present claim 1 is directed to "[a] method for increasing the content of one or more selected amino acids in a selected tissue or organ of a plant comprising transformation of a plant with at least one recombinant nucleotide sequence construct comprising tissue or organ specific regulatory sequences driving transcription during selected stages of morphogenesis, the construct being operably linked to a chimeric nucleotide sequence comprising: (i) a nucleotide sequence encoding a carrier protein comprising a plant specific protein enabling targeted expression of an amino acid enriched protein in the selected tissue or organ of the plant; said nucleotide sequence lacking a termination codon and being fused in frame with (ii) at least one nucleotide sequence comprising a selected optimal number of codons encoding an amino acid sequence comprising a selected combination of one or more amino acid residues, wherein said optimal number of codons is selected by determining the number of codons allowing a stable translation using a cell free *in vitro* translation system; said recombinant nucleotide sequence construct enabling stable targeted expression of the selected amino acid enriched carrier

protein having a stable polyamino acid extension in the selected tissue or organ of the plant" (emphasis added).

In contrast, Hoffman discloses insertion of a duplication of a 15 kDa zein sequence into the XbaI site located within the third exon of the phaseolin gene (Hoffman, column 12, Example 3 and column 13, Example 6). The construct of Hoffman therefore differs from the presently claimed invention, in which the amino acid extension replaces the termination codon of the carrier. Further, the construct of Hoffman is not "obtainable" by the method of present claims 6-8 and 23-25, which disclose a stable amino acid extension in the C- terminal end of the carrier protein.

Applicants also agree with the Examiner that "Hoffman does not teach using a cell free in vitro translation system" as in present claim 1 (Office Action, page 7).

As Hoffman does not disclose each and every element of present claims 1, 3, 6-10, 18, and 23-25, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1, 3, 6-10, 18, and 23-25 under 35 U.S.C. 102(b) as being anticipated by Hoffman.

Claims 1, 3, 6, 9-10, 13-14, 16, 18, and 23-25 are rejected under 35 U.S.C. § 102(b) as being anticipated by Moloney (U.S. Patent No. 5,650,554). This rejection is traversed.

Applicants submit that this rejection is rendered moot, in part, by the above cancellation of claim 16.

As to the remaining claims, Moloney does not disclose transformation of a plant with a construct enabling stably targeted expression of the carrier protein having a stable

polyamino extension at the C-terminal end of the carrier protein as in the presently claimed invention. Further, as noted by the Examiner "Moloney does not teach using a cell free in vitro translation system" (Office Action, page 9) (emphasis added). In contrast to Moloney, the presently claimed invention discloses that stable translation of the amino acid extension is determined "using a cell free *in vitro* translation system" prior to the actual plant transformations (present claim 1).

As Moloney does not disclose each and every element of present claims 11, 3, 6, 9-10, 13-14, 16, 18, and 23-25, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1, 3, 6, 9-10, 13-14, 16, 18, and 23-25 under 35 U.S.C. 102(b) as being anticipated by Moloney.

Claims 1-10, 18-20, 23-25 and 53 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hoffman as applied to claims 1, 3, 6-10, 18, and 23-25 above, in view of Patten et al. (U.S. Patent No. 6,413,745), Puthigae et al. (U.S. Patent No. 6,291,666), and Josefsson et al. (Journal of Biological chemistry 262: 12196-12201 (1987)). This rejection is traversed.

Applicants submit that this rejection is rendered moot, in part, by the above cancellation of claims 2 and 53.

As discussed above, Hoffman discloses insertion of a duplication of a 15 kDa zein sequence into the XbaI site located within the third exon of the phaseolin gene (Hoffman, column 12, Example 3 and column 13, Example 6). The construct therefore differs from the presently claimed invention, in which the amino acid extension replaces the termination codon of the carrier. Further, as also noted above, Hoffman does not

disclose the construct of Hoffman is not "obtainable" by the method of present claims 6-8 and 23-25, which disclose a stable amino acid extension in the C- terminal end of the carrier protein. Please see the discussion of Hoffman above.

Applicants respectfully submit that Patten et al., Puthigae et al., and Josefsson et al., alone or in combination, do not satisfy the deficiencies of Hoffman. For example, none of these references teach or suggest an amino acid extension replacing the termination codon of the carrier protein as in the presently claimed invention.

Further support for the method of the presently claimed invention may be founding the specification, which discloses that some of the constructs containing varying numbers of the one or more amino acid codons produced "unstable ... deletion variants (miniplasmids)" (see, e.g., the paragraph bridging pages 23 and 24 of the specification). The reason for the instability may be in the inefficient translation of some of the amino acid codon-enriched sequences by plant ribosomes (see, e.g., the last full paragraph on page 34 of specification). Due to this surprising observation, the applicants analyzed the stability of the constructs possessing different amino acid cassettes using the *in vitro* translation (IVT) system before transforming the constructs into the recipient plant hosts in Examples 10 and 11. The specification discloses that translation of His codon-enriched transcripts of less than 56 His residues gave rise to products of predicted, gradually increasing molecular masses, whereas transcripts of more than 56 residues were translated inefficiently and produced smeared products of lower molecular mass.

By using the IVT system, it was also demonstrated in Example 12 that transcripts of clones with one, two, and four cassettes of the Cys/Met-enriched sequence (MP-

Cys/Met-1x, MP-Cys/Met-2x, or MP-Cys/Met-4x containing 10, 20, or 40 Cys/Met residues, respectively) gave rise to the translation products of predicted, gradually increasing molecular masses. Similarly, the instability of the constructs encoding glycine or lysine extensions could be determined before performing the actual plant transformations (see, e.g., Examples 13 and 14)

In the same way, it is possible to determine the optimal number and combination of any amino acid codons, by inserting varying numbers of amino acid codons into the construct comprising the nucleotide sequence encoding the carrier protein and select constructs providing stable results in the IVT system. As demonstrated in the Examples in the specification, the increase in the length of polyamino acid sequence may lead to decreased stability of transcripts. In addition, the combination and arrangement of the codons encoding the amino acid extension may have an effect on stability of the transcripts. It is therefore necessary to determine the stability of the constructs before going into actual plant transformations in order to obtain stable transgenic plants with enriched content of the desired amino acid(s). As demonstrated in Examples 26 and 27 of the present specification, the method of the presently claimed invention result in transgenic plants, for example, in transgenic *Brassica campestris* and tobacco plants, in which the amount of the fusion protein product is stable over successive generations and the size of the protein product remains constant regardless of the plant generation.

As to claims 19 and 20 in particular, as noted by the Examiner "Hoffman does not teach using ... the napin promoter" (Office Action, page 7). In contrast, Hoffman only discloses phaseolin. Further Josefsson et al. merely discloses the genetic structure of

the storage protein napin in *Brassica napus*. As such, Josefsson et al. does not teach or suggest the napin promoter, much less a napin promoter of *Arabidopsis thaliana* as in present claim 20. Patten et al. and Puthigae et al. do not satisfy the deficiencies of Hoffman and Josefsson et al.

As none of the cited references, alone or in combination, teach or suggest all of the elements of present claims 1, 3-10, 18-20, and 23-25, Applicants submit that these claims would not have been obvious over the cited references. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-10, 18-20, 23-25 and 53 under 35 U.S.C. § 103(a) as being unpatentable over Hoffman as applied to claims 1, 3, 6-10, 18, and 23-25 above, in view of Patten et al., Puthigae et al., and Josefsson et al.

Claims 1-10, 13-14, 16, 18-20, 23-25 and 53 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Moloney as applied to claims 1, 3, 6, 9-10, 13-14, 16, 18, and 23-25 above, in view of Patten et al., Puthigae et al., and Josefsson et al. This rejection is traversed.

Applicants submit that this rejection is rendered moot, in part, by the above cancellation of claims 2, 16, and 53.

Applicants respectfully submit that those of skill in the art would not have been motivated to combine the cited references to construct a recombinant nucleotide sequence enabling stable targeted expression of the selected amino acid enriched oleosin carrier protein having a stable polyamino acid extension.

Moloney is silent as to the possible instability of the above constructs containing a polylysine extension at the C-terminal end of the carrier sequence. Moloney discloses construction of a plasmid, which contains 20 codons for the amino acid lysine (Moloney, page 33, right column, paragraph (h) of Additional Applications of the Invention). Moloney suggests that the construct may be used to transform *Brassica napus*. However, Moloney does not show any results of the expression of the polylysine extension. Applicants submit that none of the other cited references, alone or in combination satisfy the deficiencies of Moloney.

As such, Applicants submit that it would not have been obvious to those of skill in the art that some of the constructs, which encode a fusion product having a C-terminal amino acid extension, may produce unstable deletion variants and be inefficiently translated, or may even result in a translation product that contains a shorter amino acid extension than the product obtained with a similar construct having less codons. Thus, the presently claimed invention provides unexpected advantages over the cited references by avoiding time-consuming trial and error experiments with the whole plants.

As to present claims 19 and 20 in particular, Applicants submit that those of skill in the art would not have been motivated to replace the oleosin promoter of Moloney with a napin promoter of present claims 19 and 20. In contrast, Moloney discloses in column 6, lines 55-60 that "[t]o date these seed modifications have only been conducted using seed storage gene promoters that may have inherent limitations. Use of oleosin regulatory sequences provides an additional means by which to accomplish such modifications." Further, Moloney uses Arabidopsis oleosin promoter in the constructs of Examples 3, 4,

7-9 and 11. As Moloney teaches the unique aspects of oleosin promoters, Moloney teaches away from using promoters other than oleosin promoters.

None of the other cited references satisfy the deficiencies of Moloney. As discussed above, the other cited references, and Josefsson et al. in particular, do not teach or suggest the napin promoter, much less a napin promoter of *Arabidopsis thaliana* as in present claim 20. Therefore, Applicants respectfully submit that the rejection is improper, and should be withdrawn.

III. CONCLUSION

For at least the above reasons, Applicant respectfully submits that this application is in condition for allowance and requests favorable action thereon. If the Examiner believes that anything further is desirable in order to place this application in even better condition for allowance, the Examiner is invited to contact Applicant's undersigned representative at the telephone number listed below to schedule a personal or telephone interview to discuss any remaining issues.

In the event this paper is not considered to be timely filed, Applicant hereby petitions for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 01-2300, referring to Attorney Docket No. **108306-00024**. Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300, referencing Attorney Docket No. **108306-00024**.

Respectfully submitted,

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